The Possible Use of *Bacillus thuringiensis* Plus Chitinase Formulation for the Control of Spruce Budworm Outbreaks

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Abstract: Ten thousand acres of a balsam fir forest severely infested by *Choristoneura fumiferana* Clemens was treated with a *Bacillus thuringiensis* + chitinase formulation by means of Avenger aircraft. In the 70% of the territory that was properly sprayed mortality averaged 88% and foliage protection 70% as determined by visual observations. The results of this experiment were sufficiently good to recommend the use of *B. thuringiensis* + chitinase formulation for the control of spruce budworm outbreaks.

Infection of larvae of the most serious defoliator of conifers in Canada, *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae) by *Bacillus thuringiensis* Berliner is characterized by a typical septicemia enterotoxinosis. It was also observed that disease development and mortality produced by the bacteria were faster when spores of the bacillus penetrate into the hemolymph through the gut wall, and this penetration seems easier when the insect supports a tolerant infection by microsporidian (Protozoa) (Smirnoff, 1963). The results of laboratory tests revealed that hydrolysis of the chitin layer covering the gut walls of larvae by the enzyme chitinase increased considerably the pathogenicity of *B. thuringiensis* in larvae of *C. fumiferana* (Smirnoff, 1971, 1973).

During the spring of 1971, the *B. thuringiensis* + chitinase formulation was tested on two 100-acre plots of balsam fir severely infested by *C. fumiferana*. The results obtained were good, 93% averaged mortality was registered in the plot treated with *B. thuringiensis* + chitinase and 85% average mortality in the plot treated with *B. thuringiensis* alone. Foliage protection resulting from the treatment was particularly good: 76% of the current year's shoots were preserved in the plot treated with *B. thuringiensis* + chitinase and only 35% in the plot treated with *B. thuringiensis* alone.

However, it was found that in order to obtain definitive information on potential use of *B. thuringiensis* for control of spruce budworm outbreaks, it was necessary to resort to large-scale aerial applications using commercial equipment. Thus, in 1972, 10,000 acres of balsam fir forest severely infested by the spruce budworm were treated in the lower St. Lawrence Valley, Quebec, Canada. The present paper reports on the results obtained and discusses

the possibilities offered by *B. thuringiensis* for the control of spruce budworm outbreaks.

MATERIALS AND METHODS

During the spring of 1972, 10,000 acres of a homogeneous balsam fir stand that was severely infested by the spruce budworm were chosen for the tests. This stand was producing up to 15 cords of pulpwood per acre. Control plots were established 2 miles from the treated plots in a balsam fir stand of the same type. The larval population in the treated plots was 18.6 larvae per 18 in. branch tip, and there were 18.9 larvae per 18 in. branch tip in the control plots.

Spraying was done by means of 3 Avenger aircraft, each of which carried 650 US gallons of spray material. The aircraft were equipped with boom and nozzles and guided by Cessna pointers as commonly used for application of chemical insecticides. However, it was the first time that *B. thuringiensis* was sprayed by means of the commercial method. Spraying was done between June 4 and 7 when 5.98% of the larvae were in the second instar, 71.70% in the third instar, 21.57% in the fourth instar, and 0.75% in the fifth instar. The formulation per acre was:

Thuricide HPC concentrate	0.5 gal (US)
Polyglycol 400	0.5 gal (US)
Chevron spray sticker	0.16 oz (US)
Water	1 gal (US)
Chitinase (980 nephelemetric units)	10 mg

Spray rate was 2 gallons per acre.

Assessments of results in the field were made using the following techniques:

1. Kromekote cards (4 × 4 in.) to check deposit.
2. One hundred millimeter petri dishes with nutrient agar culture medium to check deposit.
3. Portable 9′ × 3′ cloth tables on iron stands to determine the number of fallen larvae and quantity of frass.
4. Electrostatic bacterial air sampler to determine the presence of *B. thuringiensis* spores in the air.
5. Small particle detector to determine the quantity of solid particles in the atmosphere.
6. The method of the 18-in. branch tip cut at midtree crown was used to determine larval population before and after spraying.

The same techniques were used in the control plots.

Twenty-five sample plots were selected at random throughout the area.

At each plot 5 co-dominant balsam fir trees were selected at 2-chain intervals, the first sample tree being at least 3 chains from the edge of the forest. Nine sample plots to serve as controls were made in a similarly infected stand 5 miles distant from the sprayed areas. Interfering trees and shrubs were cleared from around the sample trees to ensure even spray coverage. Numbered floor tiles were placed in the cleared area near each sample tree. Larval-drop tables, measuring 30 square feet, were placed under the crown of two trees in each plot to collect falling insects and to provide information on the progress of the disease in the budworm population and its possible effect on associated insects.

The microscopical examination and biochemical analyses required to determine the perturbation provoked by *B. thuringiensis* on the metabolism of larvae were performed in a field station.

RESULTS

Measurement of spray deposit revealed that only 70% of the area had been treated properly. The number of gallons per acre (GPA) measured by the droplet count varied between 0.0045 and 1.3237 GPA. Only the areas of the territory which received over 0.4 GPA were considered to have received a sufficient quantity of deposit. The quantity of *B. thuringiensis* colonies per square centimeter of culture medium varied between 21 and 113 col/cm^2, and counts over 50 col/cm^2 were considered as a good deposit. The deposit was lower than that obtained with the Micronair sprayers which equipped the Stearman aircraft during the 1971 experiment. In general, it was found that the boom and nozzle attachments were not able to give the high-quality deposit given by the Miconair system, a higher-deposit volume being in the larger drop-size categories.

Mortality of larvae in buds was 49.81% seven days after spraying. During the same period, an average of 105 larvae had fallen on each cloth table. The larval mortality in plots that received a sufficient amount of deposit averaged 88.2% (between 84 and 93%). Pupal mortality averaged 33.9%.

The survival of parasites was made evident by the presence of several healthy pupae of Tachiniidae on cloth tables. Similar to the 1971 operation, spores survived in the air of treated plots up to 15 days after spraying, and no particles remained in the air after spraying, indicating that *B. thuringiensis* treatments are not a source of pollution.

From visual observations it was estimated that some 70% of the current year's foliage was protected in the treated area compared to no protection in the control area. By using a statistical method proposed in 1972 by the Conservation Branch, Department of Lands and Forests, Quebec, and the Department of Entomology, Faculty of Forestry, Laval University, it was established that 47% of the foliage in the treated area and 12% in the control

area remained on the trees. Although these figures seem rather low, they compare advantageously with those obtained when spraying with the chemical insecticide fenitrothion.

Five to six hundred larvae were weighed and measured periodically in treated and control plots. The results obtained revealed a reduction in the weight and length of treated larvae, and this is not unusual since it was observed that treated larvae stop feeding a few days after spraying for the following ten-day period. For example: Measurements of the weight of larvae in treated plots were .0059, .0176, and .0497 gram compared to .0073, .0410, and .0579 gram for larvae from the control, a 56% decrease. On the same days, the length of larvae from treated plots was 6.55, 10.22, and 13.91 millimeters compared to 6.92, 13.94, and 14.16 millimeters for larvae from control plots, a 26% decrease. The weight of pupae was 6.3 grams in treated plots and 7.8 grams in control plots. Fifteen days after spraying the surviving larvae started feeding again and a higher defoliation, which was still considerably lower than that in control plots, was observed.

Studies on the enzymatic activities in the hemolymph of infected living larvae and healthy larvae revealed that the larval metabolism is greatly modified during infection. The activity of the glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) did not vary; the activity of the dehydrogenases was greatly reduced [isocitrate dehydrogenase (ICDH) —from 603 to 186 mU/ml*] and that of the phosphatases increased (alkaline phosphatase—from 200 to 490 mU/ml). Furthermore, the infection provoked a strong decrease, from 4.8 to 2.7%, in the lipid reserves of the organism and a strong elevation of the rate of chloride in the hemolymph (from 36.0 to 100.5 mEq/1†). In infected pupae, and increase of transaminase activity (GOT—up to 32 mU/g) and a decrease of the dehydrogenase (from 38.8 to 11.2 mU/g) were observed. The activity of the phosphatases remained elevated (alkaline phosphatase: 185 mU/g compared to 120 mU/g in the control). In healthy pupae, a strong cholinesterase activity (49.0 mU/g) was observed, whereas it diminishes to 17.5 mU/g in infected pupae. Besides, the rate of lipids decreased by half during infection, from 5.3 to 2.9% (Smirnoff and Valéro, 1972).

The estimation of egg masses made in August revealed that in more than half of the sample points in the territory sprayed with Thuricide the number of egg masses per 100 square foot of foliage for 5 branches was under 640 masses, indicating a low defoliation in 1973. Throughout the control area the number of egg masses for 5 branches always exceeded 935 masses—an indication probably of a high defoliation in 1973.

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* 1 mU/ml or g $= \frac{1 \text{ mU mole converted substract}}{1 \text{ min} \times 1 \text{ ml or g}}$

† mEq/l = milliequivalent/liter

DISCUSSION

In Maine, Dr. J. B. Dimond, professor at the University of Maine, tested several formulations of *Bacillus thuringiensis* against the spruce budworm. In a personal communication he confirmed that only sprayings with *B. thuringiensis* + the same concentration of chitinase that we used in our tests gave good foliage protection.

Special attention should be paid to determine why Plot 1 sprayed in 1971 with *B. thuringiensis* + chitinase was practically not affected by the insect this year and why the egg count carried out in the fall of 1972 indicates a low population for 1973. Is it possible that *B. thuringiensis* treatments are able to maintain their effects on the population the following year? Does a reserve of bacterial material remain in the biotope the year following a mass introduction of bacteria? These important questions are now being studied.

It was concluded from the 1972 application of *B. thuringiensis* on 10,000 acres that this bacterial insecticide offers strong possibilities for its use in the control of spruce budworm outbreaks.

Literature Cited

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